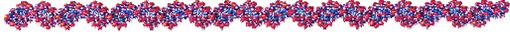




Standards, Validation, and Evaluation Studies to Aid the Forensic DNA Community



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NIJ Applied Technologies and Partnerships Conference
February 1, 2006 (Hilton Head, SC)

Disclaimers

Funding: Interagency Agreement 2003-IJ-R-029 between the [National Institute of Justice](#) and NIST Office of Law Enforcement Standards

Points of view are those of the authors and do not necessarily represent the official position or policies of the US Department of Justice. Certain commercial equipment, instruments and materials are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or endorsement by the National Institute of Standards and Technology nor does it imply that any of the materials, instruments or equipment identified are necessarily the best available for the purpose.

Our publications and presentations are made available at:
<http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm>

Presentation Outline

- Importance of Quality Results in DNA Testing
- Introduction to NIST and its Role
- Standard Reference Materials
- Validation Resources
- Evaluation and Interlaboratory Studies

Quality Is Essential in Forensic DNA Testing



Chicago Tribune

FORENSICS UNDER THE MICROSCOPE

Unproven techniques sway courts, erode justice

By Philip M. Heltman, Steve Milder and Maxine Fendley, Tribune staff reporters
Tribune executive Judith Mancini contributed to this report.
October 17, 2005



HoustonChronicle.com

Houston's leading information source

Tests find HPD's lab data wrong once again
New DNA exam indicates errors in 1997 murder case
Houston Chronicle By Ponna Khanna
February 16, 2005

- **DNA results impact lives** – the guilty can be implicated in a crime and the innocent can be exonerated
- Scientific attacks against the science behind DNA testing are rare in court now. Rather **the focus is on demonstrating that quality results were obtained.**
- **DNA databases involve comparisons** of DNA profiles analyzed at different times or in different locations

DNA Testing Requires a Reference Sample

A DNA profile by itself is fairly useless because it has no context...

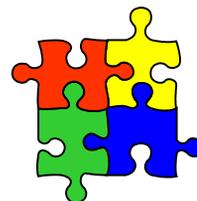
DNA analysis for identity only works by comparison – you need a reference sample



Crime Scene Evidence compared to **Suspect(s)** (Forensic Case)
Child compared to **Alleged Father** (Paternity Case)
Victim's Remains compared to **Biological Relative** (Mass Disaster ID)
Soldier's Remains compared to **Direct Reference Sample** (Armed Forces ID)

Elements for Guaranteeing Quality Results in Forensic DNA Testing

- Accepted Standards and Guidelines for Operation
- **Laboratory Accreditation**
- Proficiency Testing of Analysts
- Standard Operating Procedures
- Validated Methods
- Calibrated Instrumentation
- Documented Results
- Laboratory Audits
- Trustworthy Individuals



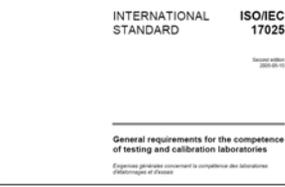
Many Labs Are Moving to ISO 17025 as Part of Their Laboratory Accreditation



ASCLD/LAB-International
An ISO/IEC Program of Crime Laboratory Accreditation
(DOE: 17025 related by ASCLD/LAB-International Requirements)
"Quality Assurance Through Assessments"

<http://www.ascl-d-lab.org/international/indexinternational.html>

a specific program of accreditation based upon the requirements of ISO/IEC 17025 – General requirements for the competence of testing and calibration laboratories



INTERNATIONAL STANDARD ISO/IEC 17025
General requirements for the competence of testing and calibration laboratories

Requires measurement traceability to national measurement standards from a National Metrology Institute

ISO/IEC 17025

5.6 Measurement traceability

5.6.1 General

All equipment used for tests and/or calibrations, including equipment for subsidiary measurements (e.g. for environmental conditions) having a significant effect on the accuracy or validity of the result of the test, calibration or sampling shall be calibrated before being put into service. The laboratory shall have an established programme and procedure for the calibration of its equipment.

ISO/IEC 17025

5.6.2.1.2 There are certain calibrations that currently cannot be strictly made in SI units. In these cases calibration shall provide confidence in measurements by establishing traceability to appropriate measurement standards such as:

- the use of **certified reference materials** provided by a competent supplier to give a reliable physical or chemical characterization of a material;

- the use of specified methods and/or consensus standards that are clearly described and agreed by all parties concerned.

Participation in a suitable programme of interlaboratory comparisons is required where possible.

NIST History and Mission

- National Institute of Standards and Technology (NIST) was created in 1901 as the National Bureau of Standards (NBS). The name was changed to NIST in 1988.

- NIST is **part of the U.S. Department of Commerce** with a **mission to develop and promote measurement, standards, and technology to enhance productivity, facilitate trade, and improve the quality of life.**

- **NIST is the National Metrology Institute for the U.S.**

- NIST supplies over 1,300 Standard Reference Materials (SRMs) for industry, academia, and government **use in calibration of measurements.**



NIST Gaithersburg Campus

Administration (Building 101)



Located in Gaithersburg, Maryland, on approximately 234 hectares (578 acres) just off Interstate 270 about 25 miles northwest of Washington, D.C.

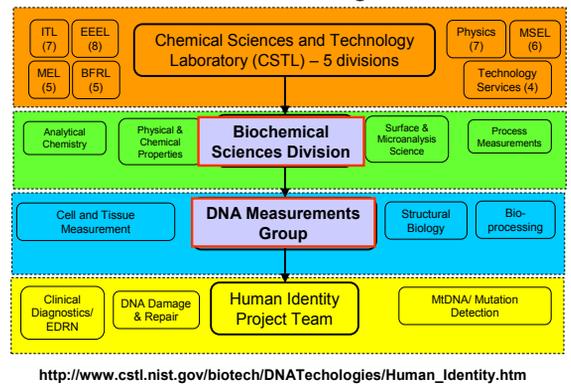
<http://www.nist.gov>

~2,500 staff



Advanced Chemical Sciences Laboratory (Building 227)

Overview of NIST Organization



NIST Human Identity Project Team



John Butler
(Project Leader)



Margaret Klaine



Pete Vallone



Mike Coble



Dave Duewer
Anal. Chem. Division



Jan Redman



Amy Decker



Becky Hill



Chris DeAngelis
computer programmer

Funding: Interagency Agreement 2003-IJ-R-029 between National Institute of Justice (NIJ) and NIST Office of Law Enforcement Standards (OLES)



National Institute of Justice
The Research, Development, and Evaluation Agency of the U.S. Department of Justice

Current Areas of NIST Effort with Forensic DNA

- **Standards**
 - Standard Reference Materials
 - Standard Information Resources (STRBase website)
 - Interlaboratory Studies

- **Technology**
 - Research programs in SNPs, miniSTRs, Y-STRs, mtDNA, qPCR
 - Assay and software development
- **Training Materials**
 - Review articles and workshops on STRs, CE, validation
 - PowerPoint and pdf files available for download

Congress Passed the **DNA Identification Act of 1994** (Public Law 103 322)

↓ Formalized the FBI's authority to establish a national DNA index for law enforcement purposes.

FBI's DNA Advisory Board
Quality Assurance Standards
for Forensic DNA Testing Laboratories
(October 1, 1998)



STANDARD 9.5

The laboratory shall check its DNA procedures annually or whenever substantial changes are made to the protocol(s) against an appropriate and available NIST standard reference material or standard traceable to a NIST standard.

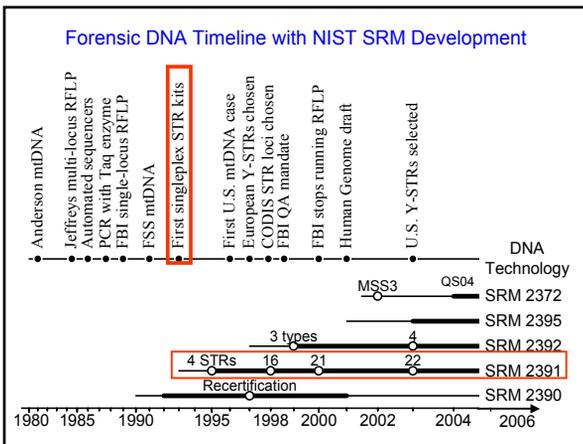


Standard Reference Materials (SRMs)



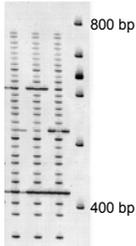
- Relevant Forensic DNA SRMs
 - SRM 2391b (DNA profiling – STRs, D1S80, DQA1/PM)
 - SRM 2392-I (mtDNA, Cell line HL-60)
 - SRM 2395 (Y-chromosome)
 - SRM 2372 (Human DNA quantitation); *in development*
- Provides national/international traceability and compatibility (aids in ISO 17025 compliance)

<http://www.nist.gov/srm>



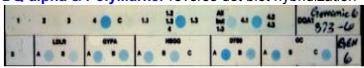
1995: SRM 2391 PCR-based DNA Profiling Standard

D1S80 Locus
Variable Number Tandem Repeat (VNTR)



800 bp
400 bp

DQ-alpha & PolyMarker reverse dot blot hybridization



6 – Pre-amplified D1S80 samples
1 – D1S80 allelic ladder
1 – size standard
10 – Genome DNA Extracts
2 – Cell line cells spotted on 903 paper for extraction



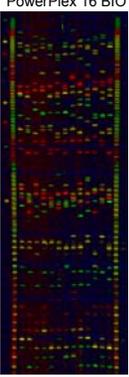
Samples originally selected to possess all DQA1 types

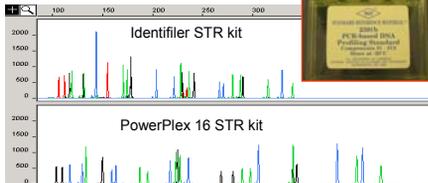
Silver stained gels for STR monoplexes

PowerPlex 16 BIO

2003: SRM 2391b released

- 22 STR Loci
- D1S80 and DQA1/PM (still certified)
- Many labs using 16plex STR kits

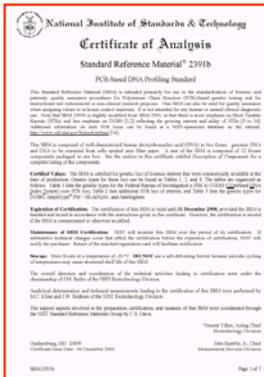




NIST SRM 2391b

All current commercial kit STR loci are certified

22 autosomal STRs characterized across 12 DNA samples



STR Locus	FES/FFS	LFL	Penta D	Penta E	D2S1338	D19S433
12,12	10,11	10,15	7,12	17,23	13,16,2	
10,11	10,11	9,11	7,12	17,26	14,16	
11,12	11,12	11,12	13,14	20,24	12,14	
10,13	10,12	8,9	5,12	17,23	11,13	
11,13	10,12	10,13	7,13	17,19	12,2,14	
11,11	10,12	9,12	12,14	23,23	12,14	
11,11*	11,12	3,2,11	12,16	17,22	13,15,2	
10,11					1,15	
10,12					0,5	
11,13					0,4	
10,12					1,5	
11,11					13,14	

Standard Reference Material® (SRM)

A NIST SRM is prepared and used for three main purposes:

- 1) to help develop accurate methods of analysis;
- 2) to **calibrate** measurement systems used to facilitate exchange of goods, institute quality control, determine performance characteristics, or measure a property at the state-of-the-art limit;
- 3) to ensure the long-term adequacy and integrity of measurement **quality assurance programs**.

The Current Task:

SRM 2372: Human DNA Quantitation Standard

Challenge:
What is a nanogram of genomic DNA ?

From interlaboratory studies we know there is a factor of 1.6 in the measurement systems currently in use. But the range is 20 fold.

SRM 2372
Human DNA Quantitation Standard

Anticipated 2006 issue

Component A: Male
Component B: Female
Component C: Mixture

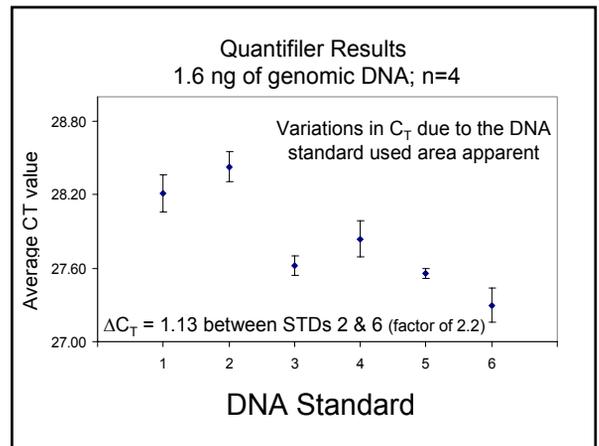
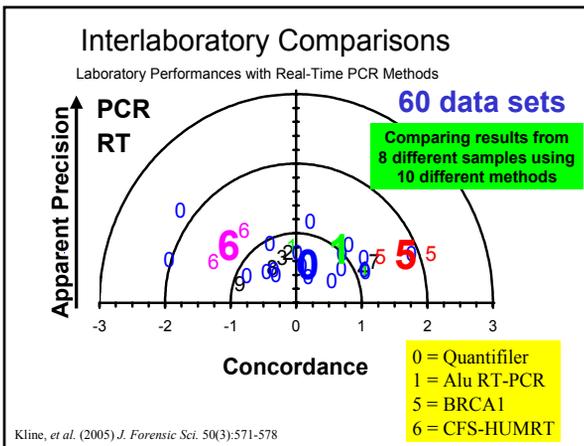
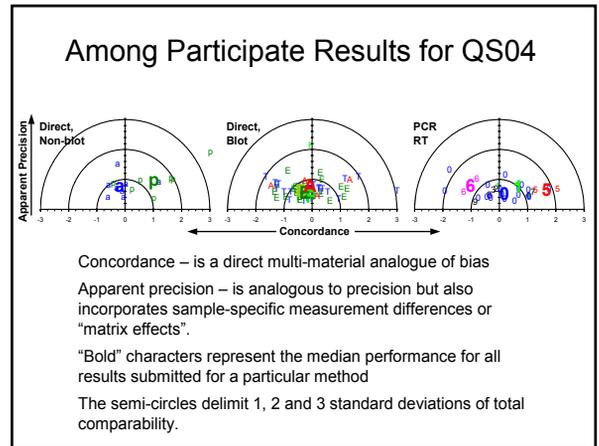
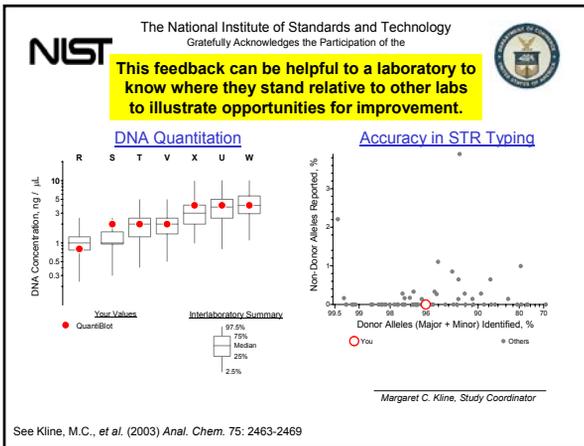
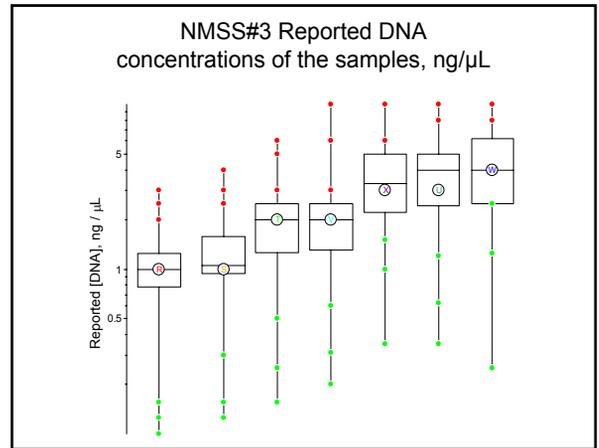
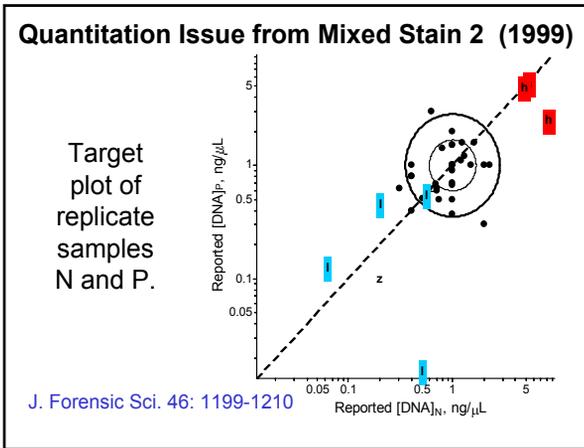
Planned Amounts: Each component 50 µL of Human Genomic DNA with a concentration targeted @ 50 ng/µL. The [DNA] for each component will be listed in the materials Certificate of Analysis.



Some of the Information Resources on the NIST STRBase Website

<http://www.cstl.nist.gov/biotech/strbase>

- [.../str_fact.htm](#) STR Fact Sheets on Core Loci
- [.../multiplex.htm](#) Multiplex STR Kit Information
- [.../y_strs.htm](#) Y-Chromosome Information
- [.../var_tab.htm](#) Variant Alleles Reported
- [.../mutation.htm](#) Mutation Rates for Common STRs
- [.../str_ref.htm](#) Reference List with ~2,500 Papers
- [.../training.htm](#) Downloadable PowerPoints for Training
- [.../validation.htm](#) **Validation Information**
- [.../miniSTR.htm](#) miniSTR Information
- [.../address.htm](#) Addresses for Scientists
- [.../NISTpub.htm](#) Publications & Presentations from NIST



Acknowledgments

Funding from interagency agreement 2003-IJ-R-029 between NIJ and the NIST Office of Law Enforcement Standards

NIST Human Identity Project Team



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This presentation available as pdf file from
<http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm>